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Environmental DNA for the surveillance of biosecurity threats in Mediterranean lagoons

Alba Ardura [a,b,*,1], Sara Fernandez [a,1], Serge Planes [c,d], Eva Garcia-Vazquez [a,b]

[a] Department of Functional Biology, C/ Julian Claveria s/n, 33006, Oviedo, Spain
[b] Centro Universitario para la Investigación y el Desarrollo del Agua (CUIDA), Edificio de Investigación del Campus de Mieres, University of Oviedo, C/Gonzalo Gutiérrez Quirós, s/n, 33600, Mieres, Spain
[c] USR3278 CRIOBE EPHE-CNRS-UPVD, 66860, Perpignan, France
[d] Centre de Recherche Insulaire et Observatoire de l'Environnement, Moorea, French Polynesia

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ABSTRACT

Invasive species that outcompete endemic ones and toxic harmful algae that cause algal blooms threaten marine resources like fisheries, aquaculture, and even tourism. Environmental DNA (eDNA) metabarcoding can help as a method for early alert. In this study, we have analyzed communities inhabiting six lagoons within the Gulf of Lion (northwest Mediterranean Sea) with spatial protection as RAMSAR and Natura 2000 sites. Employing the COI gene as the only metabarcode, we found 15 genera that have caused recognized algal bloom outbreaks in the studied lagoons since 2000. In addition, seven alien invasive species that can pose risks to the rich marine resources of the zone and lagoons were also found. The results found from eDNA are consistent with events of toxic algae blooms before and after the sampling moment and with reported occurrences of the invasive species in nearby Mediterranean areas. Multivariate multiple analysis showed the importance of anthropic pressure in the abundance of these nuisance species. Mitigation actions and routine eDNA metabarcoding in zones of special interest like these fragile French Mediterranean lagoons are recommended for early alert of nuisance species in order to plan timely management actions.

1. Introduction

Invasive species are one of the biggest biosecurity threats to marine resources and biodiversity (Molnar et al., 2008; Arthington et al., 2016). Simberloff et al. (2013) pointed out the deep transformation of the ecosystem caused by invasive species through trophic cascades and changed nutrient cycling, and how they modify the habitat in varied ways. Because of their characteristics, anthropogenic uses, and environmental circumstances, some regions are especially vulnerable. The Mediterranean Sea is considered a hotspot of biological invasions (Zenetos et al., 2012), due to its relatively low productivity and high anthropogenic stress (Occhipinti-Ambrogi and Savini, 2003). Species introduced through the Suez Canal – also called Lessepsian migration-are changing the native biodiversity (Galil, 2007; Giangrande et al., 2020). The process has accelerated following engineered expansions of the canal, which are increasing the rate of introduction of non-native organisms in Mediterranean waters (Galil et al., 2015). In addition to Lessepsian migration, other causes of the introduction and increase of invasive species in the Mediterranean are shipping and aquaculture. Aquaculture was the source of 64 introduced species, either as commodities or as stowaways (Katsanevakis et al., 2014). Approximately 1000 species are estimated to have been introduced in Mediterranean ecoregions (Zenetos et al., 2010, 2012; Tsirintanis et al., 2022), with more than half having established populations (Galil et al., 2016; Zenetos et al., 2017; Zenetos and Galanidi 2020). They have exhibitied an accelerating rate of establishment success in recent years (Zenetos et al., 2022). The intense maritime traffic that connects this sea with adjacent waters in the Atlantic and the Indian oceans would be the cause of the introduction of 308 species (Katsanevakis et al., 2014). An example is the western Atlantic blue crab *Callinectes sapidus,* which was likely introduced via ballast water (Nehring, 2011). Moreover, the introduction rate is far from saturation, and invasions are expected to continue increasing (Seebens et al., 2017), even current research show evidence about how NIS have mostly increased their presence on

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* Corresponding author. Department of Functional Biology, C/ Julian Claveria s/n, 33006, Oviedo, Spain.
E-mail address: arduraalba@uniovi.es (A. Ardura).

[1] Co-first authorship.

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Mediterranean coasts during the last 20 years, mainly aided by human-mediated activities, such as maritime transport and aquaculture (Png-Gonzalez et al., 2023).

Another threat to marine resources is harmful algae (toxic algae, algal blooms) that can also be introduced via shipping (e.g., Ardura et al., 2020). In north European waters, fish mortalities due to harmful algae cause substantial economic and social costs for the fish farming industry, while toxin syndromes associated with dinoflagellate (subphylum Myzozoa) outbreaks affect the bivalve aquaculture industry (Karlson et al., 2021). Focusing on the Mediterranean Sea, where a few potentially toxic species are suspected to have been introduced (Zingone et al., 2020), harmful algal blooms are favored by high nutrient inputs caused by human activities which produce eutrophication in many coastal areas (Tsikoti and Genitsaris, 2021). Toxicity-related events caused by some species of the dinoflagellates *Dinophysis* and *Alexandrium* impact mainly on aquaculture. Besides, the massive development of microalgal biomass reduces the coastal water quality and impacts tourism, which is an important economic resource in the region (Zingone et al., 2020). On the other hand, together with agriculture, aquaculture-derived nutrients are a cause of eutrophication in coastal and marine waters (Kang et al., 2021; Streicher et al., 2021), although in the Mediterranean Sea, the nutrient input from aquaculture seems to be very low, at least in comparison with agriculture and other sources (Karakassis et al., 2005; Morata et al., 2012), shellfish aquaculture is the cause of the main flow of introduced macrophytes in the Mediterranean (Boudouresque et al., 2020). Together with the eutrophication, the environmental factors, like temperature, pH or dissolved oxygen, and poor environmental conditions in different lagoons promote the development of harmful epibenthic dinoflagellates, such as *Ostreopsis* spp. Previous studies have shown that its proliferation may not be related to the nutrients, but rather more globally to climate change, considering the temperature and the poor environmental conditions (Ben Gharbia et al., 2019).

In their recent review, Lacoste et al. (2023) have identified the impacts of invasive species as a major, but overlooked, anthropogenic driver of biodiversity loss in Mediterranean lagoons, where most research has focused on eutrophication. Naturally stressed environments, like Mediterranean lagoons (Barnes, 1980), are even more easily colonized by alien species (Occhipinti-Ambrogi and Savini, 2003). These fragile ecosystems are today at risk of disappearing under the current sea rise due to climate change; in addition, there is also persistent pollution and eutrophication due to excessive anthropogenic uses and pressures (Soria et al., 2022). Many of these lagoons are within the RAMSAR Convention, the Habitat and Birds Directives, and the European Union Water Framework Directive (WFD) call for public action to improve their ecosystem state, which is implemented by each country according to their national legislation (De Wit et al., 2020). Protected areas (PAs) play a critical role in global biodiversity conservation. They have been created as places with a conservative role in global biodiversity, with reduced anthropogenic uses, and they have consequently experienced a pronounced expansion over time (Watson et al., 2014; Visconti et al., 2019). As places with reduced anthropogenic activities, it is expected that the coastal zones that benefit from spatial protection figures contain less invasive species than those not protected (Ardura et al., 2016). However, these protected areas are typically embedded in highly human-dominated landscapes and adopt a more inclusive conservation approach that permits human activities, which are recognized as an integral part of ecosystem functioning (Baquero et al., 2021). They are targets for aquaculture and often host fish and shellfish farms even when they are protected (Ayllón et al., 2022). Aquaculture is a widely recognized source of invasive species, due to escapes of the cultured species or to inadvertent transfers of stowaway species with seed or adult interchanges between farms (e.g., Carlton, 2003; McKindsey et al., 2007; Grosholz et al., 2015). As explained above, it is also a source of invasions in the Mediterranean Sea, although not as intense as shipping at the whole basin level (Katsanevakis et al., 2014). Vulnerable Mediterranean lagoons present a hypertrophic state (Souchu et al., 2010), which makes these environments suitable for many ecological services, such as exploitable breeding and feeding areas for fish and birds, or the production of fish and seafood (Newton et al., 2018; Maneas et al., 2019; El Mahrad et al., 2020). Based on these relevant functions, lagoons have long been exploited for human activities. In addition to these hypertrophic conditions, extreme seasonal events, water stagnation, and anthropogenic pressures can cause the degradation of these important and vulnerable ecosystems, triggering algae blooms and consequent dystrophic crises in the lagoons (Kanaya et al., 2007; Magni et al., 2008; Zheng et al., 2020). They, therefore, offer an opportunity to understand the still unexplored interaction between spatial protection, lagoon uses like aquaculture and shipping, and the frequency of nuisance species.

In this scenario, the early detection of biosecurity threats is presented as an essential tool for their successful management. Environmental DNA (eDNA) metabarcoding, where DNA is directly extracted from water or sediment samples and the species present are identified after high throughput sequencing and comparison of the obtained sequences with reference databases, has been proposed as an alert system (Borrell et al., 2017). Although metabarcoding is relatively expensive for routine analysis, targeting only one generalist gene is cheaper than sequencing multiple metabarcode regions. If the gene has relatively good coverage of taxonomic groups and geographical regions in databases, as is the case of the COI gene (Weigand et al., 2019), it may be sufficient for purposes of early detection. This has been clearly demonstrated in the Bay of Biscay where alien invasive species (AIS) were initially detected from metabarcoding and further studies demonstrated their expansion, like *Ficopomatus enigmaticus* (Muñoz-Colmenero et al., 2018) and *Crepidula fornicata* (Miralles et al., 2019).

Metabarcoding has also served to detect different nuisance species in a Mediterranean lagoon (Suarez-Menendez et al., 2020). Although the common DNA barcode, the mitochondrial cytochrome *c* oxidase I (COI–5P) gene, has been shown to discriminate between diatom species (Evans et al., 2007), the universality and quality of the sequence are lower than those of other markers (Moniz and Kaczmarska, 2009). However, overall diatom coverage was very low: 15% of all species have one rbcL or 18S sequence (Weigand et al., 2019). Therefore, although previous studies suggest the use of a small fragment of rbcL to discriminate diatom species (Macias et al., 2015), given that a single marker is proposed, considering the universality of the COI as barcode (Weigand et al., 2019) and that our objective is to detect nuisance species in general, the use of the latter is proposed for the development of this study.

The main objective of this work will be to assess the value of eDNA and COI metabarcoding barcode tools to determine the lagoons' environmental health:

a. Giving an overview of lagoon biota detected from eDNA metabarcoding,
b. Analyzing the relation between anthropogenic activities and the presence of nuisance and exploited species.

Therefore, the hypotheses to test were: **i)** *eDNA metabarcoding will detect a frequency of nuisance species (HABs and AIS) proportional to the number and intensity of anthropogenic pressures occurring in each lagoon*; **ii)** *less protected lagoons will contain a higher proportion of nuisance species*. Species present in lagoons of the northwestern Mediterranean Sea (Gulf of Lion) with different spatial protection status and coastal uses were analyzed from eDNA metabarcoding on water samples. AIS and potentially harmful algal blooms (HABs) were identified from eDNA sequences, and communities were characterized in terms of total taxonomic diversity, proportion of HABs, and proportion of AIS.

While it is true that numerous articles have been published on the usefulness of eDNA analyses in aquatic biomonitoring, this work analyses the usefulness of a simple and unique metabarcoding barcode (COI gene) as an early detection tool for nuisance species, including invasive species and HABs, in a simple, fast, accurate, and economical way.

2. Material and methods

2.1. Lagoons studied and sampling procedures

In Languedoc-Roussillon, Gulf of Lion (France), six coastal lagoon systems were sampled: Canet-Saint Nazaire, Leucate, La Palme, Bages-Sigean, Thau, Prevost-Mejean (Table 1). All of them are semi-enclosed systems connected with the Mediterranean Sea (Fig. 1); the lagoons with interconnected water bodies will be considered single units here. Details about their ecology, environmental status, and species assemblages can be found in Soria et al. (2022). Of relatively recent formation in geological time, they were traditionally employed for fishing and harbors, and also for fish and bivalve aquaculture (Cataudella et al., 2015). After serious eutrophication for decades, the diversion of treated effluents offshore reduced nutrient inputs notably, and some lagoons changed phytoplankton communities moving towards re-oligotrophication (Le Fur et al., 2019; Leruste et al., 2016, 2019). An example is the Thau lagoon, where considerable efforts were made to improve the water treatment plant systems in the lagoon watershed between 1970 and 2000. Subsequently, a decrease in nutrients was observed, followed by oligotrophication (Derolez et al., 2020).

The lagoons studied are touristic areas, with marinas and recreational navigation in all of them except La Palme, and one industrial port (Port La Nouvelle) in Bages-Sijean. Professional fishing is practiced in all the lagoons (Table 1). They have spatial protection for their ecological interest, all being considered Special Protected Areas within the Natura 2000 network, some are RAMSAR sites, and some are located in regional natural parks (Soria et al., 2022, Table 1). In Leucate, Thau, and Prévost-Mejean there is aquaculture, although it was interrupted in the latter due to bad water quality in 2019 (Table 1). The least disturbed is the relatively smaller La Palme lagoon, while Bages-Sijean, Thau (intensive aquaculture, in restoration now) and Prévost-Mejean have more anthropogenic pressure and reportedly bad environmental conditions (Table 1). These three lagoons and Canet-Saint Nazaire are more urbanized than La Palme and Leucate (Table 1; https://pole-lagunes.org/les-lagunes/), urbanization being a factor that may promote AIS that attach to artificial substrates (e.g., Airoldi et al., 2015). Details on the biotic communities reported for these lagoons can be found in Hervé and Puslé (1980, 1981), Suarez-Menendez et al. (2020), and other relevant literature (Jones et al., 2022). Details on the uses, environmental state, and spatial protection are in De Wit et al. (2020) and the webs of Conservatoire du Littoral and Pôle-Relais Lagunes Méditerranéennes (https://www.conservatoire-du-littoral.fr/and https://pole-lagunes.org/les-lagunes/cartographie-interactive/, accessed May 2023).

Harmful algal blooms in these French Mediterranean waters are monitored for the prescriptive closure of aquaculture and fishing in the affected areas. The frequency of declared outbreaks of toxic microalgae and closure days has been substantially constant over the last two decades (Fig. 2). In these years a total of 187 outbreaks were recorded (70 phytoplankton blooms and 117 episodes of seafood toxins), causing 5819 days of aquaculture closures. These data have been taken from the Harmful Algal Event Database (HAEDAT), within the International Oceanographic Data and Information Exchange of UNESCO (Intergovernmental Oceanographic Commission of UNESCO, 2021), and are openly available at http://haedat.iode.org/advancedSearch.php (accessed on May 2023). They correspond to France, Region 11(MED), grids FR-12 and FR-13.

Water samples from these lagoons were taken in November–December 2016, in the coordinates described in Table 1. Six liters of surface water were collected in three sterile bottles of 2 L each (three replicates) from the location coordinates given in Table 1. Bottles were washed before and after sampling with 10% bleach and rinsed thrice with sterile water. The water from each bottle was vacuum filtered through several filters of different materials and pore sizes to collect all DNA from the sample without clogging the filters. Details of the sampling conditions have been published in Suarez-Menendez et al. (2020).

Table 1

Lagoon systems considered in this study. French Department where they are located, geographical coordinates of sampling sites, spatial protection, type of species exploitation in each site, and lagoon characteristics. The Ramsar Convention on Wetlands of International Importance Especially as Waterfowl Habitat, as "Ramsar"; Spatial Protection Area (Birds Directive) and Spatial Conservation Area (Habitat Directive) within EU Natura 2000 Network, as SCA/SPA. From Cataudella et al., (2015), De Wit et al. (2020), Soria et al. (2022).

Lagoon	Department	Coordinates	Spatial protection	Resources/Activities	Characteristics/Problems
Canet-Saint Nazaire	Pyrenées orientales	42°39′25’’N/ 3°01′40’’E	SPA Natura 2000	Fisheries/Tourism Fishing port Marinas/Recreational navigation Agriculture Urbanization	Shallow mesohaline lagoon Eutrophication
Leucate	Aude	42°53′13’’N/ 3°02′51’’E	RAMSAR SPA Natura 2000	Fisheries/Tourism Recreational navigation Aquaculture: fish, mussels, shrimp	Deep lagoon Algal blooms Spatial use conflicts (navigation)
La Palme	Aude	42°56′20’’N/ 3°01′36’’E	RAMSAR SPA Natura 2000 La Narbonesse Natural Park	Fisheries/Tourism Salt production industry	Shallow poly- and euhaline lagoon Sporadic malfunctioning of WWTP
Bages-Sigean	Aude	43°08′29’’N/ 3°00′35’’E	RAMSAR SPA Natura 2000 La Narbonesse Natural Park	Fisheries/Tourism Marinas Industrial port Urbanization	Shallow poly- and euhaline lagoon Heavy metal pollution: shellfish fishing forbidden
Thau	Hérault	43°26′18’’N/ 3°40′49’’E	SPA Natura 2000	Fisheries/Tourism Fishing ports Recreational navigation Agriculture Urbanization Intensive aquaculture production: oysters and mussels	Deep lagoon Algal blooms Periodic anoxic events In restoration
Prévost-Mejean	Hérault	43°31′15’’N/ 3°54′44’’E & 43°33′08’’N/ 3°58′02’’E	SPA & SCA Natura 2000	Fisheries/Tourism Agriculture Urbanization Fluvial/maritime traffic Aquaculture: bivalves (oysters and mussels), interrupted for bad water quality since 2019	Shallow poly- and euhaline lagoon Eutrophication Ecological degradation of wetlands

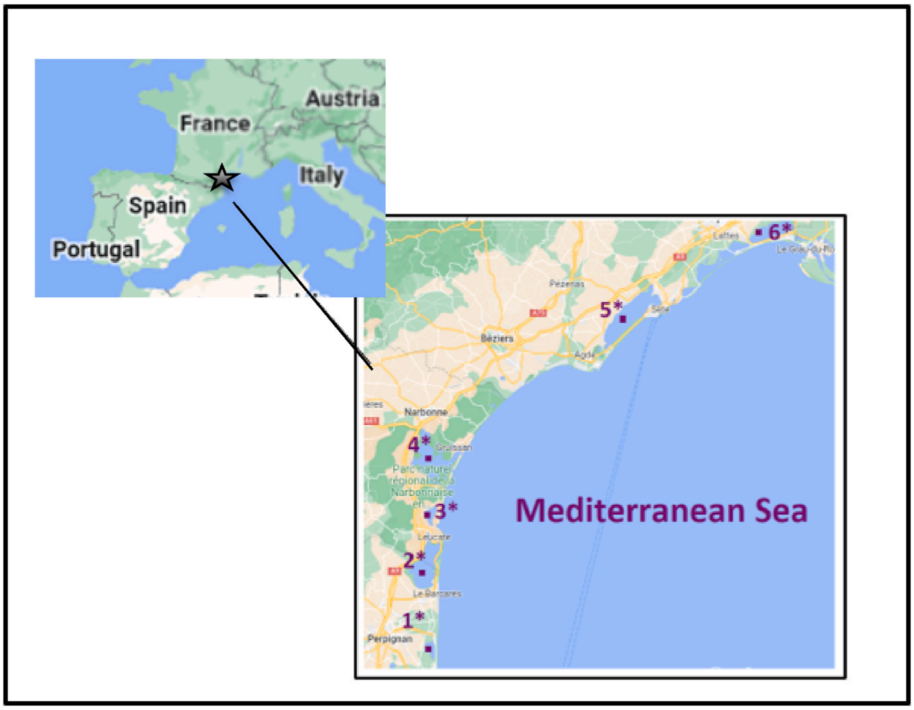

Fig. 1. Map showing the coastal lagoons sampled in the Gulf of Lion within the Mediterranean Sea: 1: Canet-Saint Nazaire, 2: Leucate, 3: La Palme, 4: Bages-Sigean, 5: Thau and 6: Prevost-Mejean. Modified by the authors from Google Maps.

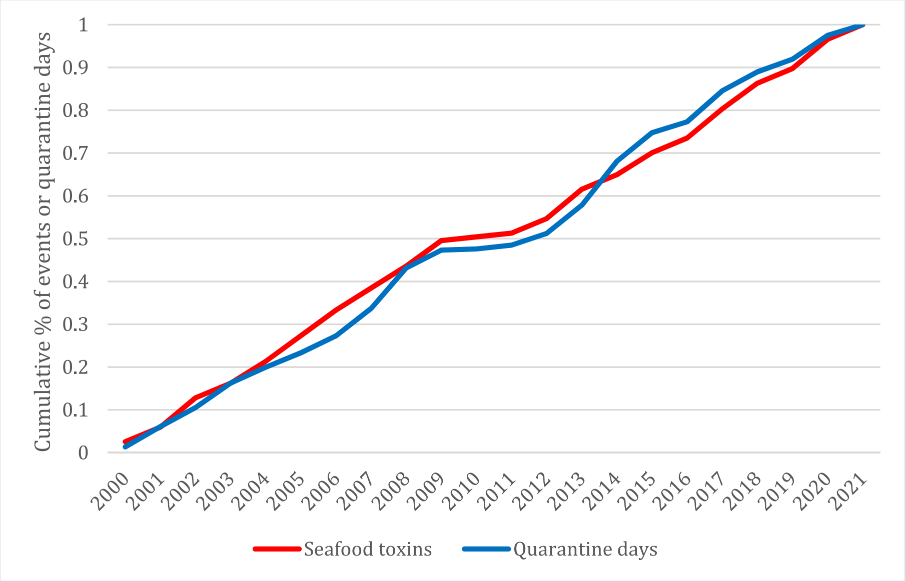

Fig. 2. Harmful algae events evolution in French Mediterranean waters over the last decades. Cumulative proportion of registered outbreaks of algae with seafood toxins, and quarantine days of closure of aquaculture and fishing. Data taken from http://haedat.iode.org/advancedSearch.php.

Briefly, the whole volume of each sample was first filtered through 10 μm nylon filters; after, each sample was divided in four and filtered through 0.8 μm polyethersulfone (PES) filters. Finally, the whole volume of each sample was pooled together and filtered through 0.2 μm PES filters. In total, 18 filters (3 replicates per 6 filters each one) were obtained per each lagoon. The filters were immediately put in clean vials with 96% ethanol, transported to the laboratory, and stored at −20 °C until analysis.

2.2. Molecular analysis

The DNA was extracted from the filters under a laminar airflow chamber to prevent contamination using the PowerWater® DNA Isolation Kit (MoBio Laboratories) following the manufacturer's protocol. The ethanol where the filters were preserved was centrifuged, and the pellet was added in the 5 mL bead tubes for the first step of the lysis process. Negative controls were included at this step to check contamination during extraction. The purified DNA was quantified by using the Qubit® dsDNA HS (High Sensitivity) Assay Kits. The concentration of each sample was checked using the Qubit® Fluorometer.

DNA extracted from the filters and pellets was sent to Macrogen (Seoul, South Korea) where it was quantified and sequenced. The eDNA was quantified by a fluorescence-based method Victor 3 (Picogreen, Invitrogen). A fragment of 313 base pairs (bp) within the cytochrome oxidase subunit 1 gene (COI) was amplified using the primers designed by Leray et al. (2013):

mlCOIintF: 5′ - GGWACWGGWTGAACWGTWTAYCCYCC-3′
jgHCO2198: 5′ - TANACYTCNGGRTGNCCRAARAAYCA-3′

The primers were modified to include Illumina sequencing adapters and sample-specific dual indices (i5 and i7) following the Illumina (2013) protocol in which the conditions for the amplicon PCR were changed to the ones described by Leray et al. (2013). The PCR reactions were undertaken by Macrogen, following the protocol Illumina (2013). After constructing the library, it was charged into the platform Illumina MiSeq v3 with reagents that generate paired-end sequences. Adapters and indices were removed from the raw data along with reads <36 bp using Scythe and Buffalo software, respectively.

2.3. Bioinformatics of HTS (High-Throughput Sequencing) data

Fastq files received from Macrogen already demultiplexed were further processed using the platform QIIME1 (Caporaso et al., 2010). All the sequence reads were assessed for quality by applying a Phred quality score threshold of 20 and were filtered by length using 200-400bp as thresholds. Merging was performed using at least 100bp overlapping and less than 15% differences. For the taxonomic assignation, Basic Local Alignment Search Tool (BLAST) alignment was performed against NCBI database of COI sequences (obtained and stored locally in 09/2017) filtered for environmental reads and using as threshold criteria: maximum E value = 10^{-50} and minimum percent identity = 97.0, which is enough in most of the cases for species identification from COI barcode (Hebert et al., 2003). When reads have multiple BLAST hits with 100% identity to different species, or the best hit has no species-level reference available, the molecular operational taxonomic unit (MOTU) was assigned to a genus level.

MOTU tables were constructed clustering reads with a 100% identity between them and maintaining all assigned sequences including singletons to retain maximum sensitivity for species detection. The removal of singletons often applied to eliminate false positives (Scott et al., 2018) was not employed here because, for marine biosecurity surveillance, a false negative is costlier than a false positive (von Ammon et al., 2018). The taxonomic information given in the reference databases for the MOTUs was checked against the World Register of Marine Species (http://www.marinespecies.org/) and AlgaeBase (http://www.algaebase.org/) as explained below.

2.4. Species taxonomy and classifications

For taxonomy nomenclature, the World Register of Marine Species WoRMS (WoRMS Editorial Board, 2022) and Algaebase (Guiry and Guiry, 2022) were followed. Only the taxonomic units identified down to a species level were considered for classification as invasive and/or harmful algae, because not all the species in a genus are invasive or harmful. The status of native or alien (alien invasive species, AIS) was determined from the Global Invasive Species Database GISD (Invasive Species Specialist Group, 2015), DAISIE inventory of alien species in Europe (Roy et al., 2020) and the Invasive Species Compendium CABI (2022), complemented with regional species lists like that of Zenetos et al. (2012).

The algae species that caused toxicity and HABs in these lagoons (Fig. 2), as determined from the IOC-UNESCO reference list (Lundholm et al., 2009 onwards), are available in HAEDAT database (Intergovernmental Oceanographic Commission of UNESCO, 2021) for most of the outbreaks, generally identified at the genus level and in some cases down to a species level. This database contains information based on yearly national reports, and the available information on individual events varies greatly from event to event or country to country. In this particular case, the data available correspond to the count of cells per liter, together with other parameters such as seafood toxin analyses, quarantine duration, pigment analysis, type of organisms affected, and the syndrome name detected.

Edible species that are harvested or fished in the region were identified from relevant regional databases and publications, described in section 2.1. They were called generically “exploited species” in this study.

2.5. Data analysis

The MOTUs assigned to a species or genus using the bioinformatics pipeline described above were taken for downstream analysis. The free software PAST v.4 (Hammer et al., 2001) was used for statistical analysis. The relationships between lagoons for the biota detected from DNA were inspected employing Non-metric Multidimensional Scaling (NMDS) on presence-absence of each MOTU (coded 1/0), with 9999 permutations and Manhattan distance. Using Manhattan distances allows us to account for species abundance and shared species absences, while the NMDS allows us to compare community composition using the rank order of the distances. The distributions of discontinuous data like the number of MOTU per taxa, were compared between samples using contingency Chi-square and pairwise post-hoc tests. The effect size was tested employing Cramer’s V.

Taxonomic diversity in each location was estimated using the Shannon-Weiner index from the total number of species of each phylum as a variable (Supplementary Table 2). Differences in diversity between lagoons were checked using t-tests.

For analysis purposes, a simplified scale was employed because all the lagoons are heavily stressed from anthropogenic activities (Table 1; Soria et al., 2022), and it is very difficult to obtain detailed data of variables that could potentially influence the number of nuisance species. This scale is measured by the intensity of aquaculture in each lagoon, the type of port present, as well as other additional stressors: agriculture, urbanization, and industrial production. The intensity of aquaculture in each lagoon was measured from the species farmed, giving 1 point to each type of species: oysters, mussels, and shrimps (maximum 3 points). The intensity of maritime traffic was expressed from the types of ports occurring in each lagoon (1 point to marinas, 1 point to industrial ports, 1 point to fishing ports — maximum 3 points). Additional disturbances that may favor HABs and AIS (agriculture, urbanization, or industrial production associated with the lagoon) were given 1 point each (maximum 3 points) (Tables 1 and 2).

Associations between biological indicators (like Shannon-Weiner diversity index, the proportion of AIS, HABs, and exploited species MOTU), and human environmental variables effects (like the intensity of

Table 2
Attribution of individual note to each identified influential or causative factor that may favor HABs and AIS and the final note attributed to each lagoon. The intensity of aquaculture in each lagoon was measured giving 1 point to each type of species farmed. The intensity of maritime traffic was measured from the types of ports occurring in a lagoon giving 1 point to each type.

	Canet	Leucate	La Palme	Bages-Sigean	Thau	Prevost-Mejean
Aquaculture	0	3	0	0	2	1
Ports	2	1	0	2	1	1
Agriculture	1	0	0	0	1	1
Urbanization	1	0	0	1	1	1
Industrial production	0	0	1	0	0	0
Sum of disturbances	**4**	**4**	**1**	**3**	**5**	**4**

aquaculture, maritime traffic, and total anthropogenic pressure as the sum of disturbances in the lagoons), were tested using Kendall rank correlation coefficient τ. Differences between groups of lagoons for mean values of diversity or other variables were tested using Student's *t* tests after confirming normality. Normality was checked using Shapiro-Wilk tests.

The effect of spatial protection and anthropogenic pressure (independent variables) on the biological indicators (exploited species, AIS, and HABs as dependent variables) was tested from multivariate multiple regression.

3. Results

3.1. Overview of lagoons biota detected from eDNA

The HTS results are available in NCBI's Sequence Read Archive repository as BioProject ID PRJNA836411 and BioSample SAMN28159850. Considering the replicates of each site together, the total number of BLAST hits at 97% identity ranged from 24,221 hits in Thau lagoon to 98,282 hits in Canet-Saint Nazaire lagoon (Supplementary Table 1). Results from Canet-Saint Nazaire lagoon have been previously published in Suarez-Menendez et al., (2020). Excluding the sequences assigned to terrestrial species and aerial fungi, the number of Molecular Operational Taxonomic Units (MOTU), or putative species, ranged between 10 in Thau to 52 in Prévost-Mejean (Supplementary Table 1). The taxonomical classification of different MOTU identified and their presence/absence in each lagoon are in Supplementary Table 3. The MOTU read counts are in Supplementary Table 3.

The taxonomic profiles of the analyzed lagoons were quite different (Fig. 3). Myzozoa (represented by dinoflagellates in these lagoons) were detected in all the analyzed samples. The proportion of Myzozoa MOTU was very high in Canet-Saint Nazaire, La Palme, and Thau, and at the same time exhibited relatively low taxonomic diversity (Table 3), suggesting eutrophication (Fig. 3). The lagoons were indeed different in their taxonomic composition ($\chi^2 = 98.9$, with 50 d.f. and $p \ll 0.001$). However, the three richer in Myzozoa (Canet-Saint Nazaire, La Palme, and Thau) were not different from each other in their taxonomic composition (post-hoc $\chi^2 = 14.3$, with 14 d.f. and $p = 0.43 > 0.05$ n.s.). In contrast, the other three more diverse lagoons (Leucate, Bages-Sigean, and Prevost-Mejean) were significantly different from each other ($\chi^2 = 38.8$ with 16 d.f. and $p = 0.001$).

The total taxonomic diversity was different in the lagoons analyzed, Shannon indices ranging between 0.79 in La Palme and 1.72 in Bages-Sijean (Table 3). The Shannon index was significantly and negatively correlated with the proportion of phytoplankton MOTU (Kendall's $\tau = -0.87$, $p = 0.014$). The mean diversity was different between the eutrophic group of lagoons (Canet-Saint Nazaire, La Palme, and Thau), characterized by high Myzozoa proportion and less diversity, and the other group (1.1 with variance 0.06 versus 1.64 with variance 0.02; $t = 4.387$, $p = 0.01$). Post-hoc comparisons between the diversity of any lagoon of the first group and any lagoon of the second group were all significant; for example, the comparison between Canet-Saint Nazaire and Leucate gave a value of $t = 4.23$ with 45 d.f. and $p = 0.001$, highly significant. The rest of the comparisons, not shown, were similar to this one.

For the similarity of species profiles among samples, NMDS analysis (small stress of 0.03, $r^2 = 0.835$ for axis 1 and $r^2 = 0.13$ for axis 2) showed the lagoons with a higher proportion of dinoflagellates MOTU (Thau, La Palme, and Canet-Saint Nazaire) connected to each other (Fig. 4). Bages-Sigean (second lowest MOTU richness), was in an independent branch of the minimum spanning tree close to Thau (the lowest MOTU richness), while the more diverse Leucate and Prevost-Mejean were located clearly apart, at a higher distance in the scatter plot (Fig. 4).

3.2. Relation of anthropogenic activities with eDNA of nuisance and exploited species

The nuisance species detected in this study are detailed in Table 4. Seven AIS (Table 4A) recognized as globally invasive in international databases (DAISIE, 2020; CABI, 2022; GISD, 2022) were found in these lagoons: three annelids, one arthropod, two tunicates, and one dinoflagellate that is also a HAB species (*Alexandrium catenella*) and can be introduced via ballast water (Lilly et al., 2002), as well as the copepod *Acartia tonsa* (Paavola et al., 2005). *Ficopomatus enigmaticus* and *Hydroides elegans* are generally transported fouling on ship hulls (de Wit, 2011; Roy et al., 2020; CABI, 2022; GISD, 2022). The most likely source of the tunicates *Ascidiella aspersa* and *Styela plicata* and the Polychaeta *Polydora cornuta* is an unintentional introduction as stowaways from aquaculture (Roy et al., 2020; GDIS, 2022). The proportion of AIS varied between lagoons from none detected in La Palme to 11.1% in Leucate (Table 3).

HABs are in Table 4B. We found MOTUs of nine of the 15 genera that have caused 119 of the 187 algal bloom outbreaks in the lagoons in the period 2000–2021 (Supplementary Table 4; http://haedat.iode.org/advancedSearch.php). The proportion of HABs over the total number of MOTU ranged between 0% in the least disturbed lagoon La Palme to 25% in Canet-Saint Nazaire (Table 3).

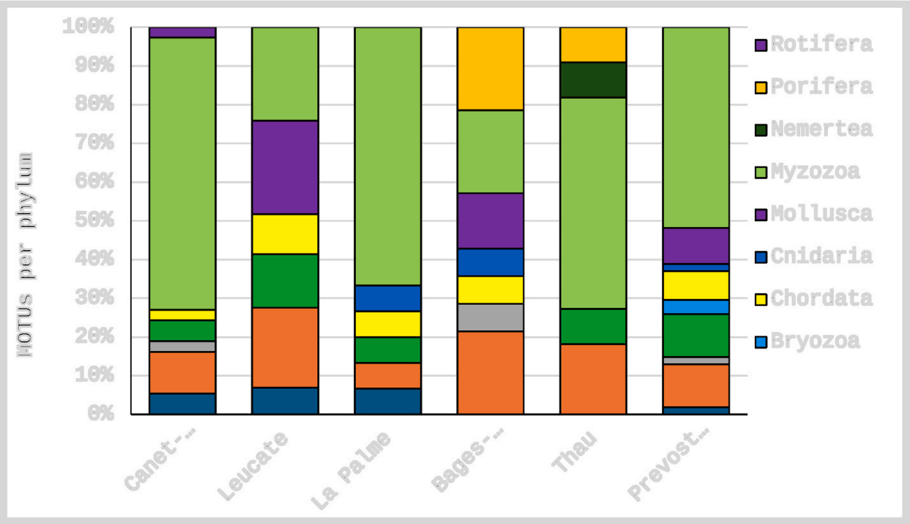

Fig. 3. Taxonomic profile of the analyzed lagoons obtained from eDNA. Results are presented as % of species of different phyla in each location.

Table 3

Biota features estimated using eDNA in the analyzed lagoons. MOTU: Molecular Operational Taxonomic Units (putative species). MOTU richness is the number of MOTU assigned to a species level, and Shannon index is Shannon-Wiener diversity index. The proportion of MOTU identified as phytoplankton, harmful algae (HABs), invasive (AIS), or exploited species is given for each lagoon.

	Canet-St Nazaire	Leucate	La Palme	Bages-Sijean	Thau	Prevost-Mejean
MOTU richness	37	29	15	14	11	54
Shannon index	1.062	1.705	0.794	1.721	1.295	1.497
% phytoplankton MOTU	0.778	0.407	0.786	0.231	0.7	0.731
% HABs	0.111	0.111	0	0.077	0.2	0.154
% AIS	0.083	0.111	0	0.077	0.1	0.058
% exploited species	0	0.185	0	0.231	0	0.096

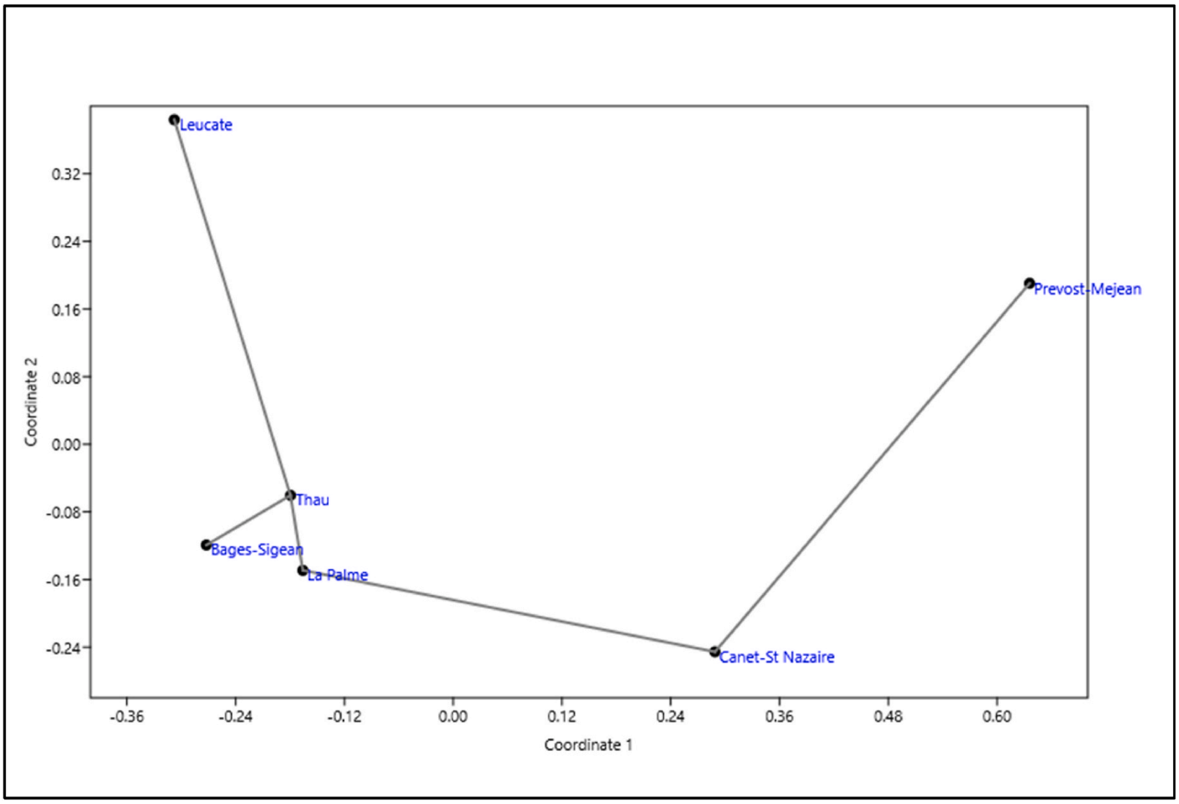

Fig. 4. NMDS scatter plot showing the analyzed lagoons. Constructed from Manhattan distances based on presence/absence of MOTU (putative species) detected from eDNA. Minimum spanning tree is presented.

The effect of the spatial protection and anthropogenic pressure was tested here with multivariate multiple regression (Table 5). These two variables are not significantly correlated to each other ($\tau = -0.59$, $p = 0.10$). The degree of anthropogenic pressure, as expected in hypothesis i), explained significantly both AIS ($r^2 = 0.757$, $p = 0.044$) and HABs ($r^2 = 0.916$, $p = 0.016$) (Table 5). However, despite expectations in Hypothesis ii) the spatial protection did not explain the proportion of AIS MOTU ($r^2 = 0.055$, $p = 0.472$ n.s.), while it significantly predicted the proportion of exploited species ($r^2 = 0.664$ with $p = 0.013$).

The proportion of AIS MOTU in the analyzed lagoons was significantly correlated with the sum of aquaculture and maritime traffic scores ($\tau = 0.86$, $p = 0.015$), and also with the sum of those and urbanization ($\tau = 0.79$, $p = 0.026$). In the same way, the proportion of HABs MOTU was positively and significantly correlated with the intensity of agriculture in these lagoons ($\tau = 0.713$, $p = 0.04$), although the correlation with aquaculture was not significant ($\tau = 0.54$, $p = 0.13$).

DNA of edible species exploited (marked with an asterisk in Supplementary Table 2) in the region by commercial or recreational fisheries appeared in these results: *Mytilus* mussels, *Cerastoderma* cockles, *Ruditapes* clams, *Dicentrarchus* sea breams, *Chelon* and *Mugil* mullets, and *Salmo* species (Supplementary Tables 2 and 3). Not surprisingly, eDNA of exploited species did not appear in the lagoons with apparent signs of eutrophication (Table 2). The proportion of MOTU of exploited species was negatively correlated with the proportion of phytoplankton MOTU ($\tau = -0.75$, $p = 0.035$), and was positively correlated with the Shannon diversity index ($\tau = 0.89$, $p = 0.012$). Diversity was also negatively correlated with the proportion of phytoplankton MOTU ($\tau = -0.73$, $p = 0.039$), but not with the proportion of AIS ($\tau = 0.46$, $p = 0.19$).

3.3. *Consistency of metabarcoding data and lagoons environmental health*

The third objective of this study was to assess the value of eDNA data for early alert of nuisance species in lagoon ecosystems. For this, results were compared with independent evidence of the species being present in the same area around the sampling time or later.

The eDNA data of this study were obtained from water samples taken at the end of 2016. Focusing on harmful algae first, the number of reads obtained for the different HABs is collected in Fig. 5, where it can be observed that *Prorocentrum* (2151 reads in total), followed by *Dinophysis*

Table 4

Nuisance species identified from eDNA in this study. **4A):** non-indigenous invasive species recognized in the Invasive Species Compendium (CABI, 2022), marked with X in the lagoons; introduction pathways recognized in GDIS, CABI, DAISIE databases are given with the most frequently reported pathway highlighted in bold. **4B):** Harmful algae causative of outbreaks in the studied lagoons (genera); those found from eDNA metabarcoding in 2016 are marked with X in the corresponding lagoons. The number of outbreaks recognized between 2000 and 2021 is given. C=Canet-Saint Nazaire, L = Leucate, LP= La Palme, B–S = Bages-Sigean, T = Thau, P-M= Prévost-Mejean.

A)

Class	Species	Invasion pathway	C	L	LP	B–S	T	P-M
Polychaeta	*Ficopomatus enigmaticus*	**Fouling**, Ballast water						X
Polychaeta	*Hydroides elegans*	**Fouling**, Ballast water		X				
Polychaeta	*Polydora cornuta*	**Aquaculture**	X					
Maxillopoda	*Acartia tonsa*	**Ballast water**	X			X		X
Ascidiacea	*Ascidiella aspersa*	**Aquaculture**, Ballast water		X				
Ascidiacea	*Styela plicata*	**Aquaculture**, Fouling, Ballast water		X				
Dinophyceae	*Alexandrium catenella*	**Ballast water**	X				X	X

B)

Genus	Outbreaks		Metabarcoding 2016					
	2000–2016	2017–2021	C	L	LP	B–S	T	P-M
Alexandrium	12	0	X				X	X
Asterionellopsis	1	0		X			X	X
Chaetoceros	8	0		X				X
Cylindrotheca	1	0	X					
Dinophysis	56	10	X					X
Gymnodinium	1	0						X
Heterocapsa	2	0						
Leptocylindrus	4	0						
Katodinium	1	0						
Nitzschia	7	0						X
Polykrikos	1	0						
Prorocentrum	3	0	X	X		X		X
Pseudo-nitzschia	18	0						
Sinophysis	1	0						
Skeletonema	21	0						X

Table 5

Multivariate multiple regression with the sum of anthropogenic pressure and the level of spatial protection as independent variables, and the proportion of AIS, HABs and exploited species MOTU as dependent variables. df = degrees of freedom.

Tests on the independent variables

	Wilks lambda	F	df1	df2	*p*
Anthropogenic pressure	0.010	32.38	3	1	0.128
Spatial protection	0.018	17.82	3	1	0.172

Tests on dependent variables

	R^2	F	df1	df2	*p*
% AIS	0.801	6.052	2	3	0.088
% HABs	0.917	16.61	2	3	0.024
% exploited species	0.903	14.02	2	3	0.030

Regression coefficients and statistics

		Coefficient	Std. error	t	*p*	R^2
% AIS	Constant	−0.051	0.051	−1.006	0.388	
	Disturbances	0.028	0.008	3.358	0.044	0.757
	Spatial protection	0.013	0.015	0.821	0.472	0.055
% HABs	Constant	−0.048	0.056	−0.852	0.457	
	Disturbances	0.046	0.009	4.957	0.016	0.916
	Spatial protection	−0.003	0.017	−0.188	0.863	0.239
% exploited species	Constant	−0.333	0.092	−3.618	0.036	
	Disturbances	0.042	0.015	2.724	0.072	0.001
	Spatial protection	0.148	0.028	5.292	0.013	0.664

(1575 reads, from which 1456 were of *Dinophysis acuminata*; see also Supplementary Table 2), and then *Alexandrium* (1070 reads in total), were the dominant HABs in the metabarcoding dataset (Supplementary Table 2).

In 2016, four outbreaks were reported from the studied lagoons, as they appear in HAEDAT database: one of PSP (Paralysis Shellfish Poisoning) caused by *Alexandrium* (*A. catenella* and *A. tamarense*), and three of DSP (Diarrhetic Shellfish Poisoning) caused by *Dinophysis* sp. (Supplementary Table 5). This was consistent with the HABs found from metabarcoding at the end of that year (Fig. 5). In the next years, period 2017–2021, a total of 28 outbreaks were detected in the studied lagoons (Supplementary Table 4), from which only 10 in 2018–2019 contain information about the causative species in HAEDAT database. The agents of those 10 outbreaks were *Dynophysis acuminata* (90%) and *Dynophysis* sp. (10%). Indeed, we cannot infer cause-effect from 2016 metabarcoding data; but the 91.4% of the *Dinophysis* reads found that

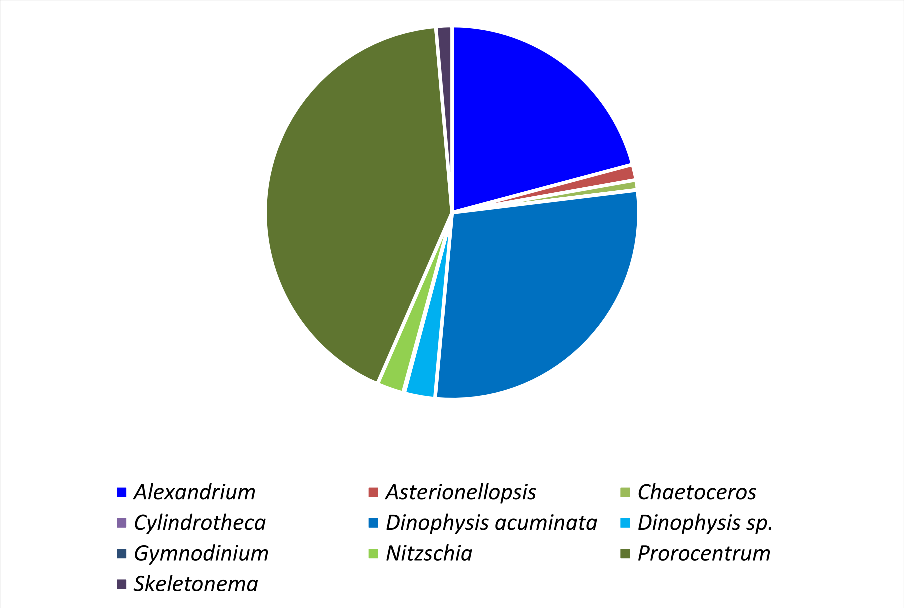

Fig. 5. Chart showing the proportion of reads of each HABs causative of outbreaks in the studied lagoons that were found from eDNA in this study. Agents of HABs outbreaks occurring on 2016 and/or 2017 are in different blue shades: *Alexandrium*, *Dinophysis acuminata* and *Dinophysis* sp.

year were precisely *D. acuminata*, which coincides with the species identified in most outbreaks (Fig. 5, Supplementary Table 5).

Regarding the AIS, we have taken as a reference the authoritative updated list of alien species established in Mediterranean waters in 2016, published by Zenetos et al. (2017). Microalgae were not considered in that list, but the AIS HABs *Alexandrium catenella*, as seen before, caused an outbreak of PSP in the same sampling year.

The copepod *Acartia tonsa* has been recorded in French Mediterranean waters since 1966 (Zenetos et al., 2017). The tubeworm *Ficopomatus enigmaticus*, was first noticed in northern France in 1921 (de Wit, 2011), and previous studies by Fornós et al., (1997) and Ardura and Planes (2017) described its presence in Western Mediterranean lagoons as well. From GISD (https://www.iucngisd.org/gisd/species.php?sc=1382, accessed June 2024), the status of this species in France is alien, invasive, established; since it occurs in France for one century, perhaps it could be considered naturalized. Four animals found from eDNA in this study were not established in the French Mediterranean in 2016: *Ascidiella aspersa*, *Hydroides elegans*, *Polydora cornuta,* and *Styela plicata*. *Ascidiella aspersa* was reported from Italy and Spain, *Hydroides elegans* and *Styela plicata* from Italy, and *Polydora cornuta* from Turkey (Zenetos et al., 2017). Nevertheless, they could have been in French lagoons at a low density without reaching the status of "established" in 2016. Some studies report the presence of some of these species in this area around that year or before, supporting the species inventory through eDNA results. *Polydora cornuta* was reported from Prévost lagoon during seasonal eutrophication crises in the 1990s (Bachelet et al., 2000); *Ficopomatus enigmaticus* individuals were found in Canet-Saint Nazaire lagoon, and *Styela plicata* in Canet port by 2014 (Ardura and Planes, 2017). This ascidian (*S. plicata*) had been already reported as a danger for oyster and mussel farming in Thau lagoon one decade ago (Davis and Davis, 2010). The other two species (*Ascidiella aspersa* and *Hydroides elegans*) would be a novelty in these lagoons.

4. Discussion

4.1. Main contributions to lagoons biosecurity

Here we have detected DNA of seven AIS, some from lagoons where they had not been described before by visual sampling, and nine HABs that have caused outbreaks of harmful algal blooms in the Mediterranean Gulf of Lion. It is worth remarking that the results presented here are based on a very simple analysis from only a few liters of water and a single metabarcode. Although we could not sample the individuals here, only their DNA, evidence of AIS in the studied zone published by other authors, together with HABs outbreaks consistent with the species found from eDNA, are solid support of the results.

As found in other studies, eDNA was able to detect non-indigenous species that are not always found in conventional surveys (Muñoz-Colmenero et al., 2018; Ardura et al., 2021; King et al., 2022). Two AIS were detected in the studied lagoons for the first time, *Ascidiella aspersa* and *Hydroides elegans*. They could have arrived through aquaculture and/or maritime traffic. They have been previously described in other Mediterranean regions, and their presence in the Gulf of Lion can be explained by the intense exchange of species in aquaculture and the high density of maritime routes in the Mediterranean basin (Coll et al., 2010).

AIS found in this study were animal species except for the dinoflagellate *Alexandrium catenella*. This contrasts with the results found in Fernandez et al. (2022) where the same methodology and genetic marker were employed. This study was developed in the neighboring Gulf of Aqaba and most of the AIS species were phytoplankton and algae. It might be that the oligotrophic conditions in the Mediterranean favor the settlement of metazoans, which represent most recognized invaders in this basin (Coll et al., 2010).

Confirming Hypothesis i) (*eDNA metabarcoding will detect a frequency of nuisance species (HABs and AIS) proportional to the number and intensity of anthropogenic pressures occurring in each lagoon*), the results of this study based on eDNA metabarcoding showed significant correlations between the proportion of nuisance species (AIS and HABs), and the intensity of their causal factors considered (aquaculture, maritime traffic, urbanization, agriculture, and industrial production) in six Mediterranean lagoons (Table 5). As highlighted by Coll et al. (2010), mariculture and vessels are the main AIS introduction vectors in the West Mediterranean. The results found here emphasize the important role of shipping in the distribution of AIS across the area. *Acartia tonsa* is typically transported in ballast water and has been inhabiting French Mediterranean waters for decades (Zenetos et al., 2017). The polychaete *Ficopomatus enigmaticus* was previously found using barcoding from Canet-Saint Nazaire lagoon by Ardura and Planes (2017), and our results suggest its distribution was wider because its eDNA was found in the

Prévost-Mejean lagoon, presumably being transported by fouling on ship hulls or commercial mollusk shells (de Wit, 2011). The intense navigation (recreational and commercial) in the zone would also explain its spread (Table 4A). The same could be applied to the Polychaeta *Hydroides elegans,* which is also transported by fouling and has been present in different Mediterranean ports for decades (Galil, 2000).

Aquaculture was the other main factor that could be related with the proportion of AIS (Coll et al., 2010), which also confirms Hypothesis i). *Polydora cornuta*, a polychaeta transported as a stowaway in interchanges between oyster farms (Clarke-Murray et al., 2014), was found in the Canet-Saint Nazaire lagoon as previously published by Suarez-Menendez et al. (2020). The ascidians *Ascidiella aspersa* and *Styela plicata* are also associated with aquaculture, and at least *S. plicata* is already a concern for aquaculture in these lagoons (Davis and Davis, 2010).

We expected HABs to be promoted principally from agriculture because nutrient effluents are more abundant from agriculture than from aquaculture in the Mediterranean (e.g., Karakassis et al., 2005; Morata et al., 2012). Our results for the proportion of HABs MOTUS confirmed this expectation, being positively and significantly correlated with the intensity of agriculture in these lagoons but not in those with aquaculture. Although only two of the nine HABs genera detected (*Alexandrium* and *Dinophysis*) had caused outbreaks in the sampling year or the following, 2016–2017, from HAEDAT database (Supplementary Tables 4 and 5), the rest had produced HABs in these lagoons in the previous period analyzed (2000–2016) and it is expected they are still there. Moreover, one of the recognized invasive species detected here from eDNA, *Alexandrium catenella*, is also a HAB-causing species. It was described as an introduced species in the Thau lagoon in 1995, likely via ballast water (Lilly et al., 2002). Although with the present analysis we cannot know if the specific strain found in these lagoons produces Paralytic Shellfish Poison or not, previous outbreaks in 2016 would confirm it. This genus is present in three of these protected lagoons, Canet-Saint Nazaire, Thau, and Prevost-Mejean, and depending on the specific strain, it could be able to induce severe harm in the environmental state.

Despite the increasing oligotrophic status of the western Mediterranean basin (Macias et al., 2015), the lagoons exhibit signals of eutrophication and periodic HAB outbreaks as seen in Fig. 2. This agrees with what was found in previous studies of the Thau lagoon or other coastal ecosystems which linked oligotrophication with an increase in potentially toxic phytoplankton species (Yamamoto, 2003; Collos et al., 2009; Derolez et al., 2020). On the other hand, other authors show evidence that the nutrient-depleted coastal ecosystems together with excess light, could favor the production of toxins by Pseudo-nitzschia (Terseleer et al., 2013), and changes in salinity could increase its allelopathic effects on cryptophytes and diatoms (Van Meerssche et al., 2018).

Logically, the sum of disturbances explained here is the proportion of both HABs and AIS that thrive in disturbed environments. The mentioned intense use of these lagoons is probably an explanation for the failure of Hypothesis ii) *less protected lagoons will contain a higher proportion of nuisance species*, at least regarding the potential effect of spatial protection on the prevention of AIS (Ardura et al., 2016). As seen above, aquaculture and shipping are directly related to the introduction of the AIS detected in our study, and it is practiced in several of these protected lagoons; similarly, recreational navigation is intense there (Table 1). In this area that has been inhabited and exploited by humans for centuries, the sum of disturbances is not negatively correlated with the level of spatial protection, thus AIS may continue spreading through shipping and aquaculture, the main AIS vectors in West Mediterranean waters (Coll et al., 2010). But this does not mean that spatial protection is useless for the improvement of environmental status and thus for the sustainability of marine resources. As we have seen in this study (Table 4), the level of spatial protection positively predicts the proportion of exploited species. As Ayllón et al. (2022) showed, not all protected areas (and not all invaders) are the same, and in this delicate lagoon environment, spatial protection should be especially encouraged.

4.2. Study limitations

The main limitation of this study was the small volume of water analyzed. A reduced number of sampling points within each lagoon was also limiting (only three except in Prévost-Mejean with six points) without temporal replicates. Although useful to monitor certain species, this impedes a complete representation of the community. Indeed, some invasive species previously described from these lagoons have not been found. For example, the blue crab *Callinectes sapidus* was reported initially from one lagoon at the east of the studied area (Berre) and rapidly expanded to the rest of the lagoons westwards (Mancinelli et al., 2017; Labrune et al., 2019), or the ctenophore *Mnemiopsis leidyi* that is present in Leucate and Bages-Sigean amongst other lagoons in the area (Marchessaux and Belloni, 2021). Likewise, other HABs reported from Mediterranean French lagoons like the ciguatera agent *Gambierdiscus*, the pinnatoxin-producer *Vulcanodinium rugosum,* and the azaspiracid-producer *Azadinium* (Zingone et al., 2020) were not found in this study. The same happened with some metazoans, for example cultured species like the Japanese oyster *Crassostrea (Magallana) gigas* that is farmed in the Prévost lagoon, its predator the gilted seabream *Sparus aurata* (Richard et al., 2020), or the abundant sand gobies of the genus *Pomatoschistus* (Berrebi and Trébuchon, 2020).

Another limitation is the genetic marker. The metabarcode used here was the COI gene, which has some limitations, for example, it is not a good marker for green algae because it contains introns. Using a barcode within the *rbc*L gene is an alternative (e.g. Bartolo et al., 2020). Thus, we may be missing information about *Caulerpa taxifolia,* already established there in 2016 (Zenetos et al., 2017), or about brown algae like *Rugulopterix okamurae*, an important invader in the area (Ruitton et al., 2021).

All the limitations shown here can be overcome by increasing the sampling volume and locations, the variety of molecular markers (depending on the species to be analyzed) and the temporal scale, so the eDNA-based tool can be improved in order to detect AIS and monitor for HABs.

Although facing many limitations, in this study we were able to use unique eDNA metabarcoding to detect nuisance species in vulnerable lagoons, so it can be considered as a proof of concept that could help further studies to develop a tool to save money and time in nuisance species monitoring efforts.

4.3. Management recommendations

Lacoste et al. (2023) emphasized the need to expand studies on AIS to understand their impact on the biodiversity of Mediterranean lagoons. In this study, we have confirmed that the technology of eDNA metabarcoding is useful for the early detection of these nuisance species. Periodical routine analysis of water samples employing this methodology could be conducted to help managers control AIS (and HABs) in these and other ecosystems. The costs are decreasing, and many biotechnological companies offer both sequencing and bioinformatic analysis, which will facilitate the practical application of metabarcoding.

Since anthropogenic pressure promotes both HABs and AIS in Mediterranean lagoons, these should be prevented. However, interrupting the human use of French lagoons is indeed unrealistic. Thus, actions to mitigate and reduce the anthropogenic pressure are recommended to reduce the spread and impact of nuisance species.

Spatial protection is encouraged to prevent further damage to these especially vulnerable ecosystems. Not only because a better environmental state favors the sustainability of the natural resources exploited there, as suggested in this study, but also because these lagoons are a historic and natural patrimony that should be safeguarded for future generations.

Finally, eDNA seems to be a great tool for the early detection of eutrophication and the control of HABs in marine and freshwater ecosystems, as recent studies have shown (Liu et al., 2020). Furthermore, designing specific markers for nuisance target species allows the development of quantitative analyses to detect potential blooms before they occur (Feist and Lance, 2021).

5. Conclusions

eDNA metabarcoding is confirmed as a useful tool for early alert of nuisance species. Here, we have detected seventeen recognized nuisance species (harmful algae and alien invasive species) from the Gulf of Lion lagoons in the western Mediterranean Sea using water samples. Two of the invasive species are reported for the first time and have likely arrived as stowaways in aquaculture (likely *Ascidiella aspersa*) and/or maritime traffic (likely *Hydroides elegans*).

Reducing anthropogenic pressure and performing routine eDNA analysis for early detection of nuisance species could help managers control their expansion. Moreover, more specific measures for the prevention of AIS, such as a higher control of the accomplishment of antifouling regulations in commercial, fishing, and recreational vessels, and a careful examination of the stocks and materials exchanged between aquaculture farms are examples that should be taken. Also, better control of agriculture runoffs would be especially useful to prevent HABs.

CRediT authorship contribution statement

Alba Ardura: Writing – review & editing, Methodology, Investigation, Conceptualization. **Sara Fernandez:** Writing – review & editing, Software, Methodology, Investigation, Formal analysis. **Serge Planes:** Writing – review & editing, Conceptualization. **Eva Garcia-Vazquez:** Writing – review & editing, Writing – original draft, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All the data are available.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2024.106601.

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